76. A method for treating a subject for an allergic or hypersensitivity condition comprising administering to the subject an effective amount of an agent selected from the group consisting of Ctx, Etx, CtxB, EtxB, or a mutant or derivative thereof, that modifies a GM1-associated activity, wherein the agent is administered with an antigen/allergen.

77. A method according to claim 76 wherein the allergic condition is selected from the group consisting of asthma, allergic cough, allergic rhinitis and conjunctivitis, atopic eczema and dermatitis, uticaria, hives, insect bite allergy, dietary allergy and drug allergies.

78. A method according to claim 76 wherein the hypersensitivity condition is contact sensitivity.

79. A method according to claim 76 wherein the agent is EtxB.

80. A method according to claim 76 wherein the agent is CtxB.

81. A method according to claim 77 which is a treatment for asthma.

82. A method according to claim 77 which is a treatment for allergic rhinitis.

REMARKS

Claims 49 to 65 were pending in this application prior to entry of the above amendments. Claims 59, 60, and 62 to 64 remain the same; claims 49, 53 to 56, 60, and 61 were amended; claims 50 to 52, 57, 58, and 65 were cancelled; and new claims 66 to 82 were added. Since the amendment adds 8 additional claims, one of which is independent, excess claim fees are due, and submitted herewith in a Fee Transmittal in addition to the two-month Extension of Time Request fee mentioned above; see the

documents accompanying this amendment. Entry and allowance of the amended claims are requested in view of these remarks.

This invention relates to agents such as Ctx, Etx, CtxB, and EtxB that bind to GM1 or modify a GM1-associated activity which are employed to treat allergic conditions such as asthma, allergic cough, allergic rhinitis and conjunctivitis, atopic eczema and dermatitis, uticaria, hives, insect bite allergy, dietary allergy and drug allergies, and hypersensitivity conditions such as contact sensitivity. A preferred embodiment provides a treatment for asthma; another, for allergic rhinitis.

It is important at the outset to reiterate the background of this invention summarized on page 9, lines 11 to 21 of the specification. Prior to the filing of this application, Applicants' research group had demonstrated that the administration of EtxB and other homologues can modulate the immune response away from the production of Th1 cytokines (*e.g.*, IFNγ) and towards the secretion of Th2 cytokines (*e.g.*, IL-4). In WO 97/02045 by Williams, *et al.*, the investigates reported results suggesting that GM1 binding agents would not find use in the treatment of allergic conditions and/or hypersensitivity conditions, since such conditions involve IgE, the production of which is generally accepted to be promoted by IL-4 and down-regulated by IFNγ. This invention is based upon the surprising finding that certain EtxB and related Etx, Ctx, and CtxB agents exhibiting special properties related to ganglioside associated activity and/or binding are, instead, efficacious in the treatment of allergic and/or hypersensitivity conditions, and have the advantage of being nontoxic and stable.

Rejections Under 35 U.S.C. § 1.112

Claims 49 to 65 were rejected under 35 U.S.C. § 112 as being indefinite because the specification does not provide sufficient enablement for using any agent that is capable of modulating a ganglioside-associated activity for treating subjects for aller-

gic/hypersensitivity conditions, and as overly broad as written. The claims have been amended in response to this rejection to particularly point out distinguishing features of the invention. Indeed, for clarification purposes, the entire claim set has been restructured to encompass four types of treatment methods, all of which use Etx, Ctx, or EtxB, or mutants or derivatives thereof having defined properties related to GM1 binding or activity modification. These are set out in independent claims 49, 56, 61, and 76. Claim 49 particularly points out methods using defined agents that bind to GM1; claim 69 points out methods that use agents that bind to GM1 which are administered with an antigen/allergen but are not coupled to it (supported in the specification on page 13 at lines 26 to 27 and onpage 18 at lines 20 to 21); claim 61, methods using defined agents that modify a GM1-associated activity such as that described in originally presented claim 51; and claim 76 points out methods employing agents that modify a GM1-associated activity administered with an antigen/allergen (as described in the specification on page 13 at lines 26 to 27).

Each of these general types may be used for allergic conditions such as asthma, allergic cough, allergic rhinitis and conjunctivitis, atopic eczema and dermatitis, uticaria, hives, insect bite allergy, dietary allergy and drug allergies distinctly claimed in dependent claims 66, 69, 73, and 77; support for the new dependent claims may be found in the specification on page 20 at lines 3 to 5 (under the table). As set out in claims 67, 70, 74, and 78, the methods may also be used to treat a hypersensitivity condition such as contact sensitivity, *e.g.*, that induced by plant poison ivy; support for the limitation of these claims may be found in the specification on page 20 at lines 6 to 7. Each method may be used with EtxB or CtxB as set out in claims 53, 55, 59, 62, 63, 71, 79 and 80. The methods are particularly efficacious in the treatment of asthma, as particularly pointed out in claims 54, 60, 64, and 81. They are also useful in the treament of allergic rhinitis, as pointed out in claims 68, 72, 75, and 82.

Since the limitations of claim 52 are recited in claims 49, 56, 61, and 76, claim 52 was cancelled. Likewise, the limitation of claim 57 was incorporated into amended

claim 49, so claim 57 was cancelled. Claims 50, 51, and 65 were cancelled without prejudice so that the four main methods claimed have dependent claims tracking the same language.

Additional enablement for the amended claim set and clarification of the written description is provided by a Declaration Under 37 C.F.R. § 1.132 accompanying this response. In it, additional data illustrating the use of an EtxB of the invention in a mouse model of asthma and allergic rhinitis are presented. The results clearly show that certain non-toxic, stable B-subunits of *E. coli* enterotoxin exhibit immunomodulatory activity that can turn off damaging inflammation in asthma and allergic rhinitis, and that this is associated with a reduction of IgE and interleukin 4 levels. Not only do these additional findings support the claims as a whole, they also underscore the patentability of claims 53, 59, 63, and 79 directed to the use of EtxBs that bind to GM1 or modify a GM1-associated activity as agents in the therapies of the invention.

Rejections Under 35 U.S.C. § 1.112

Claims 49, 51 to 53, 55 to 57, 59, and 61 to 63 were rejected under 35 U.S.C. § 103(a) as being obvious in view of WO 95/10301 by Holmgren and Czerkinsky in view of WO 97/02045 by Williams, *et al.*, and Nashar, *et al., P.N.A.S. USA 93*: 226-230, 1996. The rejection is respectfully traversed.

The latter two references report earlier work by Applicants' research group referred to above, and provide background and enablement of the invention, *i.e.*, disclosures about the properties of EtxB, supporting the patentability of the invention, not the converse, particularly, as summarized in the specification on page 7, lines 19 to 28 and page 9, lines 11 to 21), the disclosures pointed away from the invention. The Holmgren and Czerkinsky reference is also discussed in specification (on page 7 at lines 19 to 28). That publication discloses an immunological tolerance-inducing agent comprising a

mucosa-binding agent linked to a specific tolerogen (Abstract, line 1). The PCT also includes mention of allergy using a mucosa binding agent coupled to an allergen (WO 95/10301, page 1, lines 2 to 3). It is implicit within the disclosure of Holmgren and Czerkinsky that the mucosa binding agent must be coupled to the allergen. As discussed in the specification, other researchers such as Tamura, *et al.* (*Vaccine 15*: 225-229, 1997, cited on page 7 at line 23, previously cited, PTO 892) have taken directly the protocol of WO 95/10301 and tested its efficacy in preventing allergy in a murine model of Type I allergy. They reported a significant lowering of IgE levels which are a strong predictor of efficacy but they cite data, following administration of EtxB coupled to ovalbumin, which shows that EtxB was NOT effective once IgE levels are established (*i.e.*, EtxB coupled to ovalbumin was not effective in treating allergy). Thus, Tamura, *et al.*, point away from the present invention which shows that EtxB is an effective treatment in an ovalbumin (OVA) asthma model by suppressing the production of IgE antibodies even when it is not conjugated to an antigen. Furthermore, whilst Tamura, *et al.*, teach that EtxB-OVA conjugates can prevent allergy, there is no disclosure of suggestion in Tamura, *et al.*, that EtxB can work in the absence of a conjugated antigen.

It is well known that the administration of EtxB and other homologues can modulate the immune response away from the production of Th1 cytokines such as IFN-γ and interleukin 2 (IL-2) and towards the secretion of Th2 cytokines such as IL-4, IL-10 and IL-13 (see Nashar, *et al.*, and other papers by the same investigators in *P.N.A.S. USA 93*: 223-226, 1996; *Int. Immunol. 8*: 731-736. 1996; and *Immunol. 91*: 572-578, 1997, also sent to the Patent Office on 21 August 2001). IFN-γ is the classical Th1 cytokine while IL-4 is the classical Th2 cytokine. This "immune deviation" is also the basis of the disclosure by Williams, *et al.*, in WO 97/02045 (reporting earlier work by Applicants) and has been shown to be effective in the treatment of autoimmune diseases.

As summarized above, the experimental results in WO 97/02045 would suggest that GM1 binding agents, such as EtxB, would not find use in the treatment of allergic conditions and/or hypersensitivity conditions since such conditions involve IgE, the

production of which is generally accepted to be promoted by IL-4 (see for example, pages 22.2 -22.4 of "Immunology" 4th Ed (Roitt, Brostoff and Male, eds. 1996, previously cited in the file, PTO 892). Thus, the teachings in WO 97/02045 would suggest that the administration of EtxB and other homologues would upregulate the production of IL-4 from Th2 helper cells which would then promote the production of IgE. Since IgE is known to act as a mediator in an allergic response, it would be counterintuitive to use an agent such as EtxB or its homologues in the prevention and/or treatment of an allergic response or to search for agents like EtxB or its homologues useful in the treatment of allergic responses. Thus, there was a clear technical prejudice in the art before the priority date of the present invention against using an agent such as EtxB to prevent and/or treat an allergic and/or hypersensitivity condition.

Yamamoto, *et al.* (*J. Exp. Med. 185*: 1203-1210, 1997, cited in the file on a PTO-892, 21 March 2001) confirm the generally accepted wisdom in the art at the time the application was filed that agents like Ctx can induce increases in total and specific antigen specific IgE antibodies (see page 1206, col. 1 and Table 2), and these increases are associated with IL-4 production (see page 1206, col 2 and Figure 3 and commentary on page 1207, col 1). Yamamoto, *et al.*, does not disclose or suggest that agents such as CtxB, Etx or EtxB could be used in the treatment of allergy. Indeed, the results in Yamamoto, *et al.*, point away from the possible usefulness of agents such as Ctx and mutants thereof in the treatment of allergy because the results in Table 2 indicate that agents such as Ctx and mutants thereof actually promote the production of IgE antibodies which are known to be the cause of allergy.

As mentioned above, Applicants submit herewith further data to support the invention in the 37 C.F.R. § 1.132 Declaration accompanying this amendment. The data demonstrates that, contrary to the generally accepted teachings (as set out in publications such as Yamamoto, *et al.*), the EtxB subunit does not promote IgE production. Significantly and surprisingly, Applicants have demonstrated for the first time that the EtxB

subunit actually suppress the Th2 response as indicated by its effect on the suppression of IgE production.

Nashar, *et al.*, describe an assay method for measuring levels of various cellular cytokines and antibodies associated with an immune response. The disclosure in Nashar, *et al.*, relates to a comparison between EtxB and a mutant of EtxB (G33D) which does not bind to the GM-1 receptor. Nashar, *et al.*, teach that:

> EtxB in comparison with EtxB (G33D) caused an increase in the proportion of B cells, many of which were activated (CD25+); the complete depletion of CD8+ T cells; an increase in activation of CD4+ T cells; and an increase in interleukin 2 (IL-2) and an increase in interferon gamma (IFN-γ; see abstract on page 226).

Although Nashar, *et al.*, teach that IFN-γ and IL-2 can be detected in the supernatants from cultures of EtxB and EtxB (G33D) with lymphocyte populations, no IL-4, IL-5 and IL-10 could be detected in either culture. Moreover, the teachings in Nashar, *et al.*, are confined to EtxB subunit and a mutant of the EtxB subunit. In fact, Nashar, *et al.*, suggest that commercial preparations of Ctx and CtxB or purified CtxB are strongly inhibitory of lymphocyte proliferation (see page 229, right column). Thus, there are conflicting teachings in Nashar, *et al.*, in relation to the effects of Ctx and Etx on lymphocyte proliferation.

It is also clear that Nashar, *et al.*, do not disclose or suggest that:

(i) either Ctx, CtxB, Etx or EtxB might play a role in an allergic response;

(ii) the specific changes/modulating in cytokine/antibody levels which occur in an allergic response; and/or

(iii) how an assay method might be developed to identify and agent which could induce such specific changes.

Hence, alone or in combination, the references do not suggest the invention particularly pointed out in the amended claims.

Claims 50, 54, 58, 60, 64, and 65 were rejected under 35 U.S.C. § 103(a) as unpatentable over WO 95/10301, WO 97/02045, and Nashar, *et al.*, cited above, in view of Roitt, *et al.*, also cited above, and Patterson, *et al.*, (*J. Immunol. 117*: 97-101, 1996, previously cited in the file, PTO 892). Applicants submit that the rejection of claim language in claims 50and 58 related to effects on IgE-mediated responses using agents of the invention is rendered moot by the amendments presented herein, which eliminate all references to IgE.

The rejection of claims 54, 60, and 64 to 65 because they recite treatments of asthma is respectfully and emphatically traversed. The references do not disclose asthma treatments. Roitt, *et al.*, describes hypersensitivity and the four types of hypersensitivity reactions in general terms (see 19.1). Patterson, *et al.*, attempted to determine if human peripheral blood lymphocytes cultured *in vitro* could be used to study the pharmacologic effect of agents on IgE production (page 97, paragraph 1, lines 9 to 14). The other references related to the properties of Ctx and Etx have been discussed. Alone or in combination, these references are not directed to the problem of providing asthma treatments, as the Action itself acknowledges (see page 9, number 12, second sentence after the rejection). Certainly, given the multitude of papers and patents directed to asthma therapies in the medical literature, the these references wouldn't be consulted by a skilled worker looking for a new treatment. The rejection is premised on a hindsight view, for there is no suggestion in the references that Applicants' invention would be efficacious in the treatment of asthma as illustrated in the Declaration. For these reasons, then, Applicants request that the rejection be withdrawn.

Claims 49, 50 to 53, 55, 56 to 59, and 61 to 63 were rejected under the same statute as being unpatentable in view of Tamura, *et al.*, in view of WO 97/02045 or Nashar, *et al.*, all cited above. The rejection is respectfully traversed. As has been discussed, the experimental results provided by Applicants' research group in the WO 97/02045 and Nashar, *et al.*, paper, and Tamura, *et al.*, do not suggest Applicants'

claimed invention. On the contrary, they point away from it, by suggesting that GM1 binding agents would not find use in the treatment of allergic/hypersensitivity conditions.

Claims 54, 60, and 64 to 65 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Tamura, *et al.*, in view of WO 97/02045, Nashar, *et al.*, further in view of Roitt, *et al.*, all cited above. As in a rejection previously discussed, the claims are rejected for their recitation of asthma treatments. But the references are not directed to asthma. It is well established that a

> [d]etermination of obviousness can not be based on the hindsight combination of components selectively culled from the prior art to fit the parameters of the patented invention. There must be a teaching or suggestion within the prior art, or within the general knowledge of a person of ordinary skill in the field of the invention, to look to particular sources of information, to select particular elements, and to combine them in the way they were combined by the inventor.
> *ATD Corp. v. Lydall Inc.*, 48 USPQ2d 1321, 1329 (Fed. Cir. 1998, citing other Federal Ciruit cases).

> Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination. Under section 103, teachings of references can be combined *only* if there is some suggestion or incentive to do so. *ACS Hospital Systems, Inc. v. Montefiore Hospital*, 221 USPQ 929, 933 (Fed. Cir. 1984).

But there is no suggestion in the references that the agents Applicants claim would be effective in the treatment of asthma. On the contrary, the fact that immune response is so complex, the Roitt, *et al.*, reference addresses a host of pathological disorders and the cited excerpt is illustrated by an anaphylactic response to bee venom (Fig. 19.2), and both the Tamura, *et al.*, and Nashar, *et al.*, are concerned with the study of fairly narrow, sophisticated aspects of delated hypersensitivity suppression and receptor binding sites in lymphocyte subsets, respectively, together point away from the practical importance of a defined set of agents having certain properties useful in the treatment of asthma, especially since any search for a new asthma drug must be read against the vast

literature of therapeutic agents previously suggested for this problem of considerable public health importance. Applicants therefore respectfully request the rejection on this ground be withdrawn.

Alone or in combination, the cited references do not suggest the inventions set out in Applicants' amended claims. Applicants provide new ways of treating allergic and/or hypersensitivity conditions through the induction of a specific immune deviation or suppression not heretofore described. They therefore believe that they have made a new and unobvious contribution to allergic and/or hypersensitivity therapies, and respectfully request allowance of the amended claims.

Respectfully submitted,

on 12 December 2002 by

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Marked Up Version of Amendments Required by 37 C.F.R. § 1.121

49 [Amended]. A method for treating a subject for an allergic or hypersensitivity condition comprising administering to the subject an effective amount of an agent that is selected from the group consisting of Etx, Ctx, EtxB, CtxB, and mutants or derivatives thereof that bind to GM1 [capable of modulating a ganglioside-associated activity, wherein the agent is not coupled to an antigen].

53 (Amended). A method according to claim [52] 49 wherein the agent is EtxB.

54 (Amended). A method according to claim [53] 66 which is a treatment for asthma.

55 (Amended). A method according to claim [52] 49 wherein the agent is CtxB.

56 (Amended). A method for treating a subject for an allergic or hypersensitivity condition comprising administering to the subject an effective amount of an agent that is selected from the group consisting of Etx, Ctx, EtxB, CtxB, and mutants or derivatives thereof that bind to GM1 [modifies a GM1-associated activity], wherein the agent is administered with an antigen/allergen and is not coupled to an antigen.

60 (Amended). A method according to claim [56] 69 which is a treatment for asthma.

61 (Amended). A method for treating a subject for an allergic or hypersensitivity condition comprising administering to the subject an effective amount of an agent selected from the group consisting of Ctx, Etx, CtxB, EtxB, or a mutant or derivative thereof that modifies a GM1-associated activity[, and is not coupled to an antigen].